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(54) Title: COMPOSITIONS AND METHODS FOR DESIGNING AND USING COMPOSITIONS WHICH INHIBIT ACTIVATED HELPER T CELLS

(57) Abstract: Compositions containing compounds and methods for identifying and designing compounds with a basic pharmacophore which disrupts a primary activation signal in Helper T cells and induces death or anergy of activated cells are provided. Also provided are methods of using these compositions to inhibit CD4 activity and undesired immune responses which result therefrom.

Compositions and Methods for Designing and Using Compositions which Inhibit Activated Helper T Cells

Introduction

This invention was supported in part by funds from the U.S. government (NIH Grant No. NS37726) and the U.S. government may therefore have certain rights in the invention

Background of the Invention

Autoimmune conditions including, but not limited to, Multiple Sclerosis, systemic lupus erythematosus, rheumatoid arthritis and Crohn's Disease are characterized by an aberrant constellation of events resulting in clonal activation of CD-4 dependent T cells and, ultimately, a pathologic inflammatory response. Thus, while the etiologies of many autoimmune diseases are unknown, CD4-positive Helper T cells appear to be crucial mediators in both disease onset and progression (Hutchings et al. 1993).

CD4-positive Helper T cells are predominantly produced in the thymus where they undergo both positive and negative selection. Each Helper T cell produced in this organ is unique by virtue of its polymorphic T Cell Antigen Receptor (TCR) that is matched to the resident Major Histocompatibility Complex II (MHC II) proteins. The mature cells that emerge are highly diverse and selected as discriminators of self versus non-self. As these cells migrate to the periphery, they become responsive to peptide antigens presented within the groove of the MHC II heterodimer on Antigen Presenting Cells (APCs)(Brown et al. 1993). Under normal circumstances, only the Helper T cells bearing a TCR that appropriately fits with the foreign

antigen-bearing APC will become inactivated. The rest of the subset of CD4-positive cells remains quiescent. The activated T cell clonally proliferates, secreting growth factors and cytokines, and aids in the mounting of both humoral as well as cytotoxic immune responses.

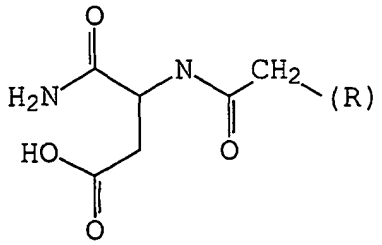
Accordingly, attempts have been made to treat the symptoms of autoimmune diseases including, but not limited to, Multiple Sclerosis, rheumatoid arthritis, systemic lupus erythematosus and Crohn's Disease, through use of compounds which inhibit CD4.

For example, U.S. Patent 5,958,882 discloses compounds which mimic the surface presented by one of five distinct lateral domains of CD4. The compounds disclosed in this patent are relatively short peptides comprising 20, or more preferably 10 to 15 amino acids derived from specific regions of CD4. These compounds are suggested to be useful in treating individuals suffering from or susceptible to conditions characterized by an undesired immune response.

Large synthetic analogs of the CDR 3-like domain of CD4 have also been established to inhibit CD4-dependent responses (Jameson et al. 1994). These large peptides lack biologically reproducible effects, however, as there is a large variation in batch-to-batch activities.

Summary of the Invention

An object of the present invention is to provide compositions comprising a basic pharmacophore of Formula (I)



for use in inhibiting CD4 activity.

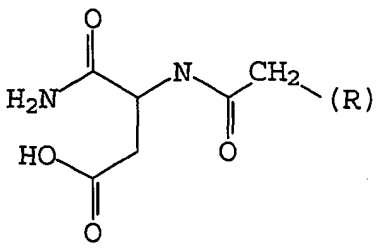
Another object of the present invention is to provide methods for designing and identifying new compounds expected to inhibit CD4 activity wherein the new compounds comprise the basic pharmacophore of Formula (I).

Another object of the present invention is to provide a method for inhibiting CD4 activity in cells or tissues comprising contacting cells or tissues with a composition comprising the basic pharmacophore of Formula (I).

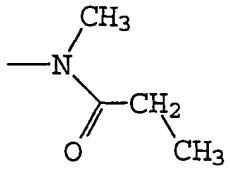
Yet another object of the present invention is to provide a method for alleviating undesired immune responses in an individual suffering from or susceptible to undesired immune responses comprising administering to the individual a composition comprising the basic pharmacophore of Formula (I).

Detailed Description of the Invention

The CD4 protein has multiple protein contacts within the activation clusters of Helper T cells. Using the crystal structures of the human CD4 protein as a template for rational design process, a basic pharmacophore has now been identified which is responsible for the CD-4 inhibitory activity of various analogs. By pharmacophore it is meant the smallest unit that retains reproducible, biological activity. The basic pharmacophore of the present invention comprises an amide group at one end, followed by a glutamic acid side chain, a nitrogen, a carbonyl, a methyl group and an R group. Modification of any of these elements except the R group was found to result in a loss of activity. The structure of this basic pharmacophore is depicted in Formula (I):



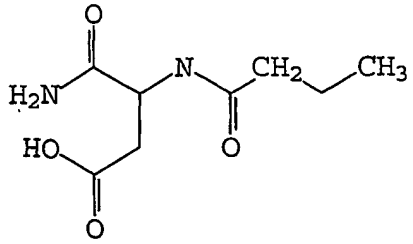
wherein R is selected from the group consisting of a straight chain alkyl, preferably 1 to 7 carbons in length, an alkyl followed by an aromatic ring preferably ranging in size from 4 to 9 carbons, or a structure of Formula (II):



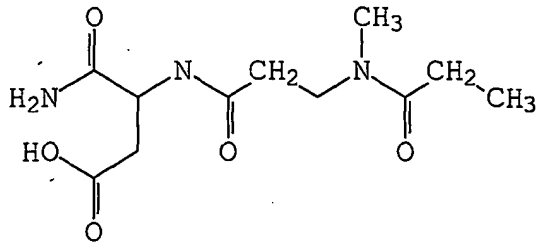
As demonstrated herein, small molecular weight (MW<500) compounds designed to comprise this pharmacophore mimic the surface of CD4 thereby disrupting the primary activation signal generated through the T cell activation cluster in the Helper T cells and inducing programmed cell death and/or anergy in the activated cells. Analogs containing this pharmacophore induce clonal deletion or anergy only in the activated sets of T cells without effecting the resting repertoire of cells needed to mount desired immune responses.

Three compounds comprising the basic pharmacophore of the present invention and different R groups were prepared. These compounds are depicted herein as Compound 1, 2 and 3.

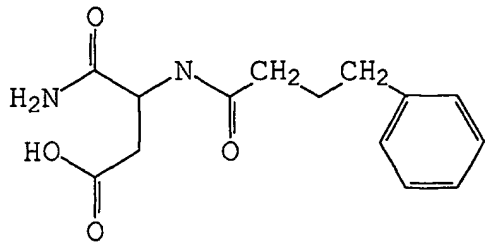
Compound 1:



Compound 2:



Compound 3:



The ability of each of these compounds to inhibit CD4 activity was demonstrated in an assay for mixed lymphocyte reaction (MLR) inhibition. These analogs showed greater inhibitory activity than the original analog disclosed by Jameson et al. 1994. In addition, Compound 1, 2 and 3 have a molecular weight of approximately 5 times less than the original analog disclosed by Jameson et al. 1994 and possess highly consistent biological activities. Of these, Compound 1 with the single methyl group attachment demonstrated the highest CD4 inhibitory activity.

The CD4 inhibitory activity of compounds comprising the basic pharmacophore of the present invention was confirmed in an *in vivo* murine model in accordance with procedures set forth by Tretiakova et al. (Nature Biotechnology September 2000 18:984-988). This model employed the use of C57/BL mice to generate a well-characterized CD4-dependent response to a murine retrovirus, MuLV. The CD-4 dependent response is used to drive the Cytotoxic T Lymphocyte (CTL) response. It is well established that inhibition of the Helper T cell response abolishes the CTL response. Accordingly, animals

of this model were administered a single intravenous bolus injection of Compound 3 after the Helper T cell response had been initially generated and then once again after re-challenge with the MuLV. After three weeks, the animals were sacrificed and the CTLs were assayed for their ability to recognize the MuLV-env protein, also referred to as target-specific killing response, and for their ability to respond to novel stimuli, also referred to as allogeneic stimulation. Only mice treated with Compound 3 showed inhibition of the virus target-specific response while still maintaining a robust allogeneic response.

Thus, provided in the present invention are compositions comprising compounds with the basic pharmacophore of Formula (I). As demonstrated herein, such compounds disrupt the primary activation signal in Helper T cells and induce death or anergy of activated cells both *in vitro* and *in vivo*. Also provided in the present invention are methods for producing other new compounds with these capabilities through synthesis of compounds with this basic pharmacophore. Methods for synthesizing such compounds can be performed routinely by those of skill in the art in similar fashion to the chemical syntheses described in Example 1 or other well known methods. In addition, the identification of this basic pharmacophore and its activities enables one of skill in the art to screen for additional compounds comprising this basic pharmacophore which are also expected to disrupt the primary activation signal in Helper T cells and induce death or anergy of activated cells both *in vitro* and *in vivo*.

Compositions comprising a compound with this basic pharmacophore are useful in inhibiting CD4 activity, and in particular undesired CD4 activity such as occurs in autoimmune diseases including, but not limited to, Multiple Sclerosis, Crohn's Disease, rheumatoid arthritis and systemic lupus erythematosus, as well as other undesired

immune responses such as occurs in tissue transplant rejection and against gene therapies. Accordingly, the present invention also relates to method for inhibiting CD4 activity in cells or tissues by contacting cells or tissues with a composition comprising a compound with the basic pharmacophore of Formula (I). In addition, the present invention provides methods for inhibiting an undesired immune response in an individual suffering from or susceptible to undesired immune responses by administering to the individual a composition comprising a compound with the basic pharmacophore of Formula (I). Doses of compounds to be administered and modes of administrations can be determined routinely by those skill in the art based upon data from assays such as described herein. Acceptable pharmaceutical formulations for these compounds can also be determined routinely by those skill in the art based upon teachings in standard reference texts such as *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA, **1985.**

The following nonlimiting examples are provided to further illustrate the present invention.

EXAMPLES

Example 1: Synthesis of Compounds 1, 2 and 3

Using established protocols in solid-phase F-moc chemistry, various target compounds were synthesized on an Advanced ChemTech 440 MOS synthesis robot. First, Rink Amide MBHA resin (Nova Biochem) was added to each reaction vessel. Next, the resin was activated using dimethyl formamide (Fisher), and then washed using piperidine (Acros). One of four amino acids was added to each of the reaction vessels such that each filled one-quarter of the vessels. Those amino acids were glutamine, glutamic acid, asparagine, and aspartic acid in the form of f-moc-Gln(trt)-OH (Advanced ChemTech), f-moc-Glu(OtBu)-OH (Nova

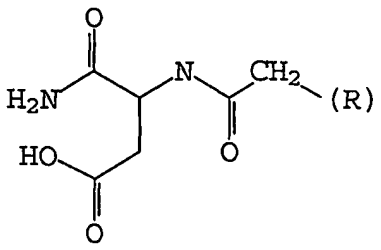
Biochem), f-moc-Asn(trt)-OH (Advanced ChemTech), and f-moc-Asp(OtBu)-OH, respectively. This was carried out in four-fold excess, relative to the resin. The amino acids were linked to the resin using diisopropyl carbodiimide (Fisher), also in four-fold molar excess. The vessels were again washed using piperidine.

The linked amino acids were then activated using dimethyl formamide. The vessels were once again washed using piperidine. One of nine compounds was then added to each of the four amino acids, yielding thirty-six unique end products. These nine compounds were added in ten-fold excess relative to the activated amino acids, and were linked using diisopropyl carbodiimide, also in ten-fold excess. These compounds were hydrocinnamic acid (Acros), propionic acid (Aldrich), 5-methoxy-1-indone-3-acetic acid (Aldrich), cyclohexylacetic acid (Aldrich), 2-cyclopentene-1-acetic acid (Aldrich), cyclopentanecarboxylic acid (Fluka), cyclohexanecarboxylic acid (Fluka), 3-phenylbutyric acid (Fluka), and 2-ethylbutyric acid (Fluka). The reaction vessels were again washed with piperidine, and then once again with methanol to wash and dry them.

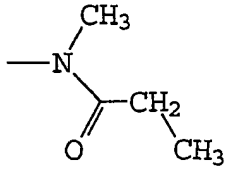
The final products were cleaved from the resin using trifluoroacetic acid (Acros). The trifluoroacetic acid was removed, and the compounds were dried with a VirTis Sentry lyophilizer. The dried products were finally resolubilized in Hanks Balanced Saline Solution (Sigma) and prepared for *in vitro* testing.

What is Claimed is:

1. A composition comprising a compound with a basic pharmacophore of Formula (I):



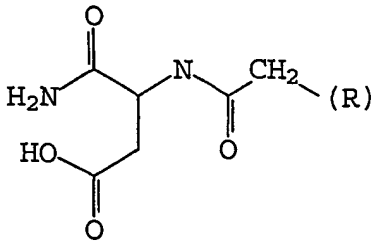
wherein R is selected from a group consisting of a straight chain alkyl, an alkyl followed by an aromatic ring, or a structure of Formula (II):



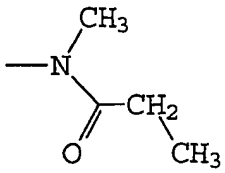
2. A method for inhibiting CD4 activity in cells or tissues comprising contacting cells or tissues with a composition of claim 1.

3. A method for inhibiting an undesired immune response in an individual suffering from or susceptible to undesired immune responses comprising administering to the individual a composition of claim 1.

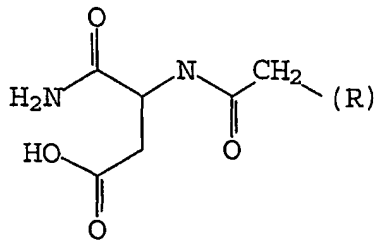
4. A method for producing a compound which disrupts a primary activation signal in Helper T cells and induces death or anergy of activated cells comprising synthesizing a compound comprising a basic pharmacophore of Formula (I):



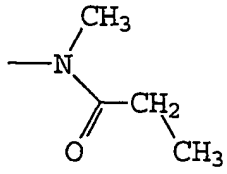
wherein R is selected from a group consisting of a straight chain alkyl, an alkyl followed by an aromatic ring, or a structure of Formula (II):



5. A method for identifying compounds which disrupt a primary activation signal in Helper T cells and induce death or anergy of activated cells comprising screening compounds for a basic pharmacophore of Formula (I):



wherein R is selected from a group consisting of a straight chain alkyl, an alkyl followed by an aromatic ring, or a structure of Formula (II):



and wherein the presence of this basic pharmacophore in the compound is indicative of the compound being capable of disrupting a primary activation signal in Helper T cells and inducing death or anergy of activated cells.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/30835

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 31/19, 31/195
US CL : 514/557, 561, 563, 564
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/557, 561, 563, 564

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST and STN:CAPlus and Registry files

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	BRAY, A. M. et al. Simultaneous Multiple Synthesis of Peptide Amides by the Multipin Method. Application of Vapor-Phase Ammonolysis. J. Org. Chem., 1994, Vol. 59, pages 2197-2203, see entire document.	1, 4, 5 --------- 2, 3
X --- Y	FERGUSON, G. et al. The Bacterial Pigment from Pseudomonas lemonnieri. Part 1, J. Chems. Soc., Perkin I, 1980, pages 1782-1787, see entire document.	1,4,5 --------- 2,3
X --- Y	WO 90/14429 A1 (NOVO NORDISK A/S) 29 November 1990 (29.11.90), especially pages 8, 20-21.	1, 4 ---------- 2, 3, 5
A	TRETIAKOVA, A. P. et al. Rational design of cytotoxid T-cell inhibitors. Nature Biotechnology. September 2000, Vol. 18, pages 984-988.	1-5

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/30835

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96/20722 A1 (THOMAS JEFFERSON UNIVERSITY) 11 July 1996 (11.07.96) pages 17-20, see entire document.	2,3,4